A Phase I Clinical and Pharmacokinetic Trial of Hepsulfam

Peter M. Ravdin, Kathy A. Havlin, Milton V. Marshall, Thomas D. Brown, James M. Koehler, John G. Kuhn, Gladys Rodriguez, and Daniel D. Von Hoff

Division of Oncology, Department of Medicine [P. M. R., K. A. H., M. V. M., T. D. B., G. R., D. D. V. H.] and Department of Pharmacology [J. M. K., J. G. K.], University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7884

ABSTRACT

Hepsulfam (1,7-heptanediol-bis-sulfamate) is one of a series of bis-sulfamate acid esters that was synthesized in an attempt to improve the antitumor efficacy of busulfan. Hepsulfam has shown broad antineoplastic activity in preclinical studies. This Phase I trial evaluated hepsulfam given as a single i.v. dose every 21–35 days. Twenty-nine patients with refractory solid tumors participated in this study. Twenty-six of these patients had had either prior chemotherapy or radiation therapy. Fifty-two courses of treatment were given at doses ranging from 30 to 360 mg/m²/day. The dose limiting toxicity was prolonged thrombocytopenia and granulocytopenia. This toxicity was cumulative with Grade 3 or 4 thrombocytopenia occurring in 3 of 15, 4 of 9, and 2 of 2 patients in the first, second, and third courses of ≥210 mg/m², respectively. This toxicity was noted in patients with ≤1 prior chemotherapeutic regimen, as well as in patients with >1 prior chemotherapeutic regimens. Nonhematological toxicities included Grade 3 or 4 nausea and vomiting and fatigue. There was no evidence of pulmonary toxicity. Plasma levels of hepsulfam were quantified by gas chromatography in 12 patients. The plasma and blood half-lives were 15.9 ± 4.6 and 90 ± 13 h, respectively. No objective tumor responses were seen. We conclude that the maximally tolerated dose when hepsulfam is given as a single dose every 35 days is 210 mg/m², but that there is significant risk of cumulative hematological toxicity at this level.

INTRODUCTION

Hepsulfam (1,7-heptanediol-bis-sulfamate, NSC 329680, sulfamin, 1,7-heptanediol ester, sulfamic acid ester) (Fig. 1) is one of a series of bis-sulfamic acid esters that was synthesized in an attempt to improve the antitumor efficacy of busulfan. Hepsulfam, like busulfan, is an alkylating agent that induces DNA cross-links and DNA-protein cross-links. Unlike busulfan, which induces predominantly DNA intrastrand cross-links, hepsulfam induces predominantly interstrand cross-links (1, 2).

Preclinical toxicity animal studies showed the major toxicity in mice, rats, and dogs to be thrombocytopenia and leukopenia. This hematological toxicity was delayed in onset (1–2 weeks), dose related, and slowly reversible. A 10% lethal dose of 330 mg/m² was established in mice (3).

Because preclinical testing suggested that hepsulfam has a broader range of action against solid tumors than busulfan, it was decided to conduct a Phase I clinical trial in patients with refractory solid tumors (4). Preclinical testing suggested that single dose schedules were as effective as multiple dose schedules, so hepsulfam was given in this trial as a single dose, once every 21 days, and when it was observed that nadir counts occurred late, every 35 days. We now report the maximum tolerated dose, toxicity profile, preliminary pharmacokinetics, and antitumor effect of hepsulfam when given by this schedule.

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2 To whom requests for reprints should be addressed, at Division of Oncology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7884

MATERIALS AND METHODS

Patient Selection. Patients with histologically documented advanced solid tumors refractory to all forms of effective therapy were candidates for this study. Other eligibility criteria were: (a) age ≥18; (b) life expectancy of ≥12 weeks; (c) a Southwest Oncology Group Performance status ≤2; (d) at least 3 weeks since last chemotherapy; (e) adequate bone marrow function (white blood cell count ≥3,000/μl, absolute neutrophil count ≥1,500/μl, platelets >100,000/μl, and hemoglobin ≥10 g %); (f) adequate liver function (total bilirubin ≤1.5 mg/dl, serum glutamic oxaloacetic transaminase ≤2.0 times normal, and normal prothrombin time); (g) adequate renal function (creatinine ≤1.5 mg/dl or creatinine clearance ≥60 ml/min) and no evidence of pulmonary fibrosis, and with forced expiratory volume in 1 s and diffusing lung capacity for CO of ≥70%. All patients had signed an informed consent prepared according to institutional and federal guidelines.

Drug Formulation. Hepsulfam for clinical use was supplied by the National Cancer Institute's Division of Cancer Treatment as a 2-component formulation. The first component was 150 mg of hepsulfam freeze-dried from 10% T-butanol in 10-ml vials. The second component was 5-ml vials and consisted of 10% ethanol, 40% propylene glycol, and 0.05 M pH 7.4 phosphate buffered saline. The hepsulfam was diluted in the second component to yield a concentration of 30 mg/ml. The drug was then diluted into 150 ml of 5% dextrose in water, and was infused over 30 min.

Study Design. Before the initiation of treatment with hepsulfam, all patients had a complete medical history and physical examination. All patients also had complete blood counts with differential, full serum chemistry panel, determination of prothrombin time and partial thromboplastin time, urine analysis, stool guaiac, electrocardiogram, chest x-ray, and scans of measurable lesions. History and physical examination, complete blood counts with differential, and complete blood chemistry were performed weekly while the patient was on study. Pulmonary function tests were obtained pre-study and with every other course of therapy. Scans of measurable lesions were obtained at a minimum of every 6 weeks, and tumor measurements were performed at least every second treatment cycle with the Southwest Oncology Group Southwest Oncology Group criteria being used for response evaluation. Toxicity was graded according to National Cancer Institute criteria.

Based on the murine 10% lethal dose of 330 mg/m², a starting dose of 30 mg/m² was selected. The originally planned dose escalation scheme was as follows: 30, 60, 100, 150, 210, 270, 360, and 450 mg/m². The first patient entered at each level was observed for 3 weeks before subsequent patients were entered. At least 3 patients were studied at each dose level. Dose escalations for individual patients were not permitted. In the original plan, the dose was to be repeated (toxicity and tumor status permitting) every 21 days. However, based on the late nadirs seen in patients, the decision was made to extend the dose interval to 35 days after entry of the first 2 patients at 210 mg/m² dose level. Patients were continued on study until tumor progression was documented or until they developed a Grade 4 hematological toxicity or Grade 3 toxicity in any other organ system.

Pharmacokinetic Studies. Blood specimens for pharmacokinetic studies were obtained on 23 of 29 patients during the first cycle of therapy. Blood samples were obtained pre-study, 15 min into the infusion, at the end of the infusion, and then 5, 10, 20, 30, 45, and 60 min later.

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Additional samples were obtained 2, 3, 4, 6, 8, 12, 24, 36, and 48 h postinfusion. An aliquot of whole blood was frozen for analysis, and the remainder was spun down and plasma aliquots frozen. Urine samples were also used in this evaluation and were obtained pre-study, over h 0–6, 7–12, 13–24, 25–36, and 37–48 postinfusion.

Hepsulfam levels were determined in physiological fluids by capillary gas chromatography after derivatization with sodium iodide (5). Hepsulfam reference standards were obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute.

Pharmacokinetic parameters were calculated using model independent methods (6). The terminal rate constant was determined by log-linear regression analysis of the terminal phase of the blood/plasma concentration-versus-time curves. The terminal blood/plasma half-lives were calculated by half-life equals 0.693/terminal rate constant. The AUC5 and the area under the first moment curves AUMC were calculated by the trapezoidal rule, with extrapolation to infinity. Clearance was calculated by dividing the total dose of hepsulfam received by the AUC extrapolated zero to infinity. The apparent Vdss was determined by the following relationship:

\[ V_{dss} = \frac{(Dose \times AUMC)/(AUC^2) - (dose \times \text{infusion time})/(2 \times AUC)}{\text{AUC}}. \]

**RESULTS**

Twenty-nine patients were entered on this trial. Patient characteristics are seen in Table 1. A total of 52 courses of treatment were administered. The median number of treatment courses was 2–3 with 14, 10, 2, and 3 patients receiving 1, 2, 3, or 4 courses, respectively. Two patients died early during the first cycle of therapy and were not evaluable for toxicity or response. Both of these patients had documented extensive metastatic pulmonary disease and died of respiratory failure at 6 and 9 days after receiving 210 mg/m² of hepsulfam. These deaths were both believed to be due to disease progression and not drug related. Data from these patients are not included in the evaluation of drug toxicity. No patient was dose escalated. One patient at the 210-mg/m² dose level received a fourth course at 210 mg/m² after having had prolonged Grade 3 thrombocytopenia with the third course.

Toxicity. The major toxicity of hepsulfam was hematological. This toxicity was delayed in onset, dose-related, slowly reversible, and cumulative (became more profound with repeated cycles). Table 2 shows the toxicity seen with the first cycle of therapy and were not evaluable for toxicity or response. Both of these patients had documented extensive metastatic pulmonary disease and died of respiratory failure at 6 and 9 days after receiving 210 mg/m² of hepsulfam. These deaths were both believed to be due to disease progression and not drug related. Data from these patients are not included in the evaluation of drug toxicity. No patient was dose escalated. One patient at the 210-mg/m² dose level received a fourth course at 210 mg/m² after having had prolonged Grade 3 thrombocytopenia with the third course.

Table 3 and 4 show hematological toxicity in the second and third cycles. During these cycles, hematological toxicity was more prolonged with Grade 3 or Grade 4 thrombocytopenia seen in 3 of 8 patients during the second cycle at 210 mg/m² and in the only patient at 270 mg/m² level. Prolonged cytopenias were again noted with 4 of 8 patients at 210 mg/m² and the only patient at 270 mg/m², taking ≥42 days to recover. Both patients receiving a third cycle of therapy at 210 mg/m² experienced Grade 3 or 4 thrombocytopenia with recovery times of

\[ V_{dss} = \frac{(Dose \times AUMC)/(AUC^2) - (dose \times \text{infusion time})/(2 \times AUC)}{\text{AUC}}. \]
Table 4 Hematological parameters Cycle 3

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>No. of patients</th>
<th>WBC</th>
<th>Granulocytes</th>
<th>Platelets</th>
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<tr>
<td>60</td>
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<td>6.1</td>
<td>4.2</td>
<td>269</td>
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<tr>
<td>150</td>
<td>2</td>
<td>6.3-8.4</td>
<td>3.7-6.3</td>
<td>168-568</td>
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<tr>
<td>210</td>
<td>2</td>
<td>1.8-2.1</td>
<td>0.7-0.7</td>
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</tbody>
</table>

Hematological toxicity

Patients with toxicity (Grade 2, 3, or 4)

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<th>Granulocytes</th>
<th>Platelets</th>
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<td>0.00</td>
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<tr>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>210</td>
<td>1.10</td>
<td>0.20</td>
<td>0.11</td>
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No./µl × 10⁹.

Fig. 2. Nadir platelet counts in the first and subsequent courses in patients receiving >210 mg/m² hepsulfam. O, Patients who had received 0 or 1 prior chemotherapeutic regimen; □, patients who had received >1 prior chemotherapeutic regimen.

Fig. 3. Nadir granulocyte counts in the first and subsequent courses in patients receiving >210 mg/m² hepsulfam. O, Patients who had received 0 or 1 prior chemotherapeutic regimen; □, patients who had received >1 prior chemotherapeutic regimen.

Table 5 Plasma pharmacokinetic parameters for hepsulfam

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>No. of patients</th>
<th>Peak concentration (µg/ml)</th>
<th>Half-life (h)</th>
<th>Clearance (liters/h/m²)</th>
<th>Vd (liters/m²)</th>
<th>AUC (µg/h/ml)</th>
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<tr>
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<td>15.9</td>
<td>7.0</td>
<td>54</td>
<td>51.2</td>
</tr>
</tbody>
</table>

Although there is no preclinical evidence for hepsulfam induced pulmonary toxicity, because of documented cases of busulfan pulmonary toxicity, patients had pulmonary function tests prestudy and then after treatment. No consistent pattern of changes was evident in these data. At the 210 mg/m² dose level after 1 cycle of therapy for the patients for whom data were available, forced expiratory volume in 1 s changed +1, +2, +3, and −5%, and diffusing lung capacity for CO +6, +3, and −19% from pretreat values.

Nonhematological toxicities were not dose limiting in this study. Grade 1 and 2 nausea and vomiting, and fatigue were noted in 4 of 29 and 6 of 29 patients, respectively. These toxicities were not clearly dose related. There was one case of what was initially believed to be hepatotoxicity (Grade 3 elevation of alkaline phosphate, serum glutamic oxaloacetic transaminase, and lactate dehydrogenase, but not total bilirubin).

Review of the patients' abdominal computer tomographic scans, however, showed that the increase in hepatic enzyme levels coincided with progression of preexisting hepatic metastases.

Antitumor Activity. There were no objective tumor responses among the 5 patients with measurable disease, or no regression of the tumor in the 24 patients with evaluable disease who participated in this trial.

Pharmacokinetics. Levels of hepsulfam were quantitated in plasma, blood, and urine by capillary gas chromatography with electron capture detection. The deviation products from busulfan and hepsulfam are 1,4-diiodobutane and 1,7-diiodoheptane, respectively. Blood samples were collected for 23 patients for hepsulfam analysis. Quantitative analyses have been performed on 15 of the 23 patients. Hepsulfam concentrations in blood have been determined for 8 patients and in plasma for 15 patients. In the blood, plasma, and urine, we detected a peak that elutes between the 1,4-diiodobutane and 1,7-diiodoheptane, which was confirmed by gas chromatography-mass spectroscopy to be IH. Preliminary inspection of the profile for IH in blood and plasma indicates that they are similar. Similar plasma levels for IH were observed at the 100- and 270-mg/m² dosage levels at the end of infusion. In the urine, larger amounts of IH were observed. The IH is present in the 0–6-h collection with peak concentrations observed in the 12–24-h samples.

The plasma pharmacokinetic results are summarized in Table 5. The preliminary plasma concentration versus time curves for patients receiving hepsulfam at levels from 100 to 270 mg/m² are illustrated in Fig. 4. Peak plasma and blood levels occurred shortly after the infusion ended. There was a linear relationship between the peak plasma concentrations and dose (r = 1.0, P < 0.01), as well as for the AUC and dose (r = 0.94, P < 0.02). A positive correlation between the dose and peak blood concentration was also observed (r = 0.98, P < 0.01), but a dose-related change in the AUC in whole blood was not apparent.
The peak blood to plasma ratio decreased with increasing dose, then remained fairly constant beyond 150-mg/m² at an average blood/plasma ratio of 2.6%. The harmonic mean plasma half-life was 15.9 ± 4.6 h compared with a terminal half-life of 90 ± 13.5 h in blood. The average plasma clearance of hepsulfam was 6.0 ± 1.7 liters/hr/m² compared with the mean clearance of 0.09 ± 0.04 4 liters/h/m² in blood. The apparent steady state volume of distribution decreased in plasma from 130 to 51 liters/m² over the same dosage range. Urinary excretion of unmetabolized drug during the first 48 h was 23 ± 9.3%.

**DISCUSSION**

We have conducted a Phase I trial of hepsulfam, which is an analogue of busulfan, but has a broader spectrum of activity against solid tumors. This study was conducted by giving Hepsulfam as a single I.V. infusion every 21 or 35 days. The major objective of this study was to evaluate the toxicities of hepsulfam and to establish a dose to be used in future clinical trials.

The dose limiting toxicity of hepsulfam, like busulfan, is hematological. This toxicity appeared cumulative in that count nadirs became lower with each cycle of therapy. On the first cycle of therapy, no Grade 3 or greater hematological toxicities were seen. At doses of ≥210 mg/m², thrombocytopenia was reversible, but recovery delayed taking ≥5 weeks. During subsequent cycles, hematological toxicities were more severe and recovery times more prolonged. The 2 patients treated at 210 mg/m² for a third cycle developed Grade 3 and 4 thrombocytopenia, which required more than 100 days to recover. This prolonged thrombocytopenia occurred in 2 patients who had only received one prior treatment regimen (5-FUra and 5-FUra-leucovorin), and in only one of which had prior radiotherapy (to the mediastinum). Thus the apparent dose limiting toxicity of hepsulfam given as a single dose every 35 days depends on the number of cycles given. It is clear from this trial that the dose for multiple cycles of therapy should be ≤210 mg/m². The hematological toxicity profile of hepsulfam is similar to busulfan, which also can cause a slowly reversible myelosuppression that may last for long periods.

Nonhematological toxicity in this trial was mild and non-dose-limiting. One patient did develop a Grade 3 elevation in liver enzymes that did not normalize. However, the review of this patient's abdominal computer assisted tomographic scans suggests that this was probably due to the progression of preexisting hepatic metastasis. No clear evidence was seen for pulmonary toxicity in this trial, but the lack of pulmonary function tests for a number of the patients and the large variability in the numbers obtained make it impossible to accurately assess whether hepsulfam might cause a cumulative pulmonary toxicity.

No objective responses were seen in this trial, but the limited number of courses given to a few patients with a wide variety of tumor types precludes any conclusions as to the antitumor efficacy of hepsulfam.

The pharmacokinetics of hepsulfam are different than for busulfan. In contrast to busulfan’s plasma half-life of 2–3 h (7), the terminal plasma half-life for hepsulfam is 16 h. Differences in the metabolic fate and distribution of these compounds may explain the differences in their kinetic profiles. The pharmacokinetic properties of hepsulfam observed in patients was very similar to that suggested by preclinical studies in beagles and mice (5). A prolonged half-life was observed in red blood cells compared with plasma.

Glutathione plays a major role in the metabolic disposition of busulfan (8), whereas hepsulfam has been shown not to react with glutathione (4). The metabolic disposition of hepsulfam is still unknown. We were unable to detect any metabolic or breakdown products of hepsulfam other than 7-ido-1-heptanol, which has been identified as the major hydrolysis product from *in vitro* derivatization rather than *in vivo* metabolism (9).

From binding studies with plasma and whole blood, we observed that hepsulfam is bound primarily to red blood cells. Our observations agree with these reported by Brodfluehrer and Powis (10) who reported that at 10 mg/ml, all of the hepsulfam was bound to red blood cells. The blood to plasma ratio for busulfan was reported by Ehrsson and Hassan (11) to be 1.05 compared with our finding of a ratio of 2.6 for hepsulfam. After ultrafiltration, we could not detect any hepsulfam in the protein-free ultrafiltrate when hepsulfam was added to whole blood at a concentration of 16 mg/ml. This preferential binding could account for the observed decrease in the apparent Vₐ while in plasma with increasing dose and a corresponding increase in Vₐ with dose for whole blood. The pharmacokinetic properties of hepsulfam observed in patients was very similar to that suggested by preclinical studies in beagles and mice (5). A prolonged half-life was observed in red blood cells compared with plasma.

Based on the results of this study, the recommended Phase II dose of hepsulfam is 150 mg/m², with careful hematological monitoring and cessation of the drug for Grade 3 or greater hematological toxicity or for platelet recovery times of ≥35 days. The evidence of cumulative toxicity suggests that hepsulfam will be a difficult drug to give on a 21- or 35-day schedule. The drug might be worth exploring further in the setting where autologous marrow rescue would allow larger doses to be given with less concern about cumulative bone marrow failure.

**REFERENCES**


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